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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No. : 09/925,139 Confirmation No.: 3066

Applicant : Crooke et al.

Filed: : August 8, 2001

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Examiner : J. Schultz

Customer No. : 36441

Title : ANTISENSE MODULATION OF CHOLESTERYL ESTER TRANSFER PROTEIN EXPRESSION

JDS Considered Aug. 31, 2004

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR §1.132

I, Dr. Susan Freier, a citizen of the United States, residing at 2946 Renault Street, San Diego, CA 92122, DO state the following:

1. I hold a B.A. in Mathematics (1972) from Carleton College, Northfield, Minnesota and a Ph.D. in Chemistry (1976) from the University of California, Berkeley, California.

2. I have been employed by Isis Pharmaceuticals, Inc. (hereinafter "Isis"), for about fourteen years. Isis, the assignee of the above-identified patent application,

specializes in oligonucleotide technology and uses the latest in bioinformatics programs to identify sites on selected genes for oligonucleotide screening.

I am presently the Executive Director of antisense lead identification at Isis and am responsible for a number of projects. I lead a project utilizing antisense oligonucleotides for functional genomics of novel targets, including the use of computational genomics to characterize target RNAs and their variants, rapid throughput screening to identify active antisense oligonucleotides for novel targets, and Q-RT-PCR and microarrays for expression analysis. I also lead a project for determining microRNA function in mammals, including the use of computational identification of miRNAs and miRNA targets and the use of functional genomics to characterize miRNA biology and identify therapeutic applications of modulation of miRNA activity. In addition, I lead a group charged with the identification and characterization of novel mechanisms for antisense oligonucleotides, including the use of computational genomics to identify mRNA variants, alteration of RNA processing, evaluation of siRNA and miRNA mechanisms. Another project I am responsible for involves biophysical and biochemical evaluation of novel antisense oligonucleotides, including the evaluation of thermodynamics and kinetics of hybridization to oligonucleotide and large structured targets, evaluation of the biochemical properties of novel oligonucleotides, characterization of antisense activity in cell assays, and protein-oligonucleotide binding.

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3. During the course of my employment at ISIS, I performed and supervised experimentation employing oligomeric compounds and inhibition of mRNA expression. My work has involved designing assays to screen oligomeric compounds against specific genes as well as interpreting the results from such assays. I have authored or co-authored numerous scientific journal articles regarding the same. I am an expert in the art of antisense technology and oligonucleotide screening. A copy of my *curriculum vitae* is attached as Exhibit 1.

4. I have reviewed the Office Action dated March 16, 2004.

5. This Declaration is filed to submit comments on statements in the Office Action regarding the motivation for combining the cited references and the alleged reasonable expectation of success by one of skill in the art for inhibiting the expression of any particular gene or mRNA with oligomeric compounds based only upon a given gene sequence.

6. It is currently not possible to predict before the appropriate experiment is performed on any particular target, which experiments will generate oligomeric compounds that will have a significant level of inhibition of target expression.

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7. One skilled in screening of oligomeric compounds cannot, *a priori*, reasonably expect a significant level of inhibition of a gene or mRNA simply because methods of screening oligomeric compounds are available and/or routine. The statements in the Office Action regarding reasonable expectation of success are neither accurate nor capable of being supported.

8. Each gene is unique. For instance, if one skilled in the art achieved at least 50% inhibition in the expression of a first gene with oligonucleotides that are specific to the first gene, one skilled in the art **would not** reasonably expect success in achieving at least 50% inhibition in the expression of a ***different*** gene with a different set of oligomeric compounds that are targeted to the different gene or mRNA. The level of inhibition of expression that is observed for one target has no bearing on the level of inhibition of expression expected for a different target.

9. Taylor et al., *1999* Drug Disc. Today, 4(12):562 (hereinafter "Taylor") is a review article that makes unsupported assertions about the ease of identifying target sites on ***any*** gene for oligonucleotides that, upon binding to the target, can inhibit gene expression. The determination of target sites on a gene that permits one to identify suitable, highly inhibitory oligonucleotides for that gene is not a process that can be predicted to be easy or simple, based merely upon the identification of the gene

sequence of the target gene or even a suggestion that inhibition of a particular gene may be desirable.

10. Taylor also purports that only 3-6 oligonucleotides need to be screened in order to find an oligonucleotide that inhibits expression with 66-95% efficiency. Taylor, however, has numerous deficiencies that seriously impact its ability to teach one skilled in the art how to screen for such active oligonucleotides. For example, Taylor fails to identify the chemical modifications that make the oligonucleotides reported therein chimeric. In addition, Taylor fails to identify any bioinformatics program reported therein (particularly the one that apparently can screen as few as 6-9 oligonucleotides to find one that inhibits gene expression with 66-95% efficiency) by name. Indeed, I am not aware of any such computer program. Further, Taylor fails to identify the manufacturer of such a bioinformatics program reported therein. Taylor, rather than actually teaching sufficient details that would actually allow one skilled in the art to carry out screening methods with such fantastic results, simply refers to "unpublished data." Taylor also fails to teach how such results may be attained manually. Thus, Taylor acts only as general guide for screening oligomers and does not provide any details sufficient for one skilled in the art to carry out any particular methodology. Indeed, I am unaware of any algorithm or methodology presently available that would enable one either to predict *a priori* with such confidence whether a

particular level of inhibition of a gene or mRNA will occur.

11. As one of skill in the art and as an author of over 75 scientific references, the mere fact that Taylor published in a peer-reviewed journal *does not* mean that Taylor teaches how to practice that which it purports to teach.

12. Neither Baracchini et al., US Patent No. 5,801,154 (hereinafter "Baracchini") or Bennett et al., US Patent No. 5,955,443 (hereinafter "Bennett"), taken individually or together, compensate for the many deficiencies discussed above in relation to Taylor. Baracchini and/or Bennett fail to teach how to select target regions for the 3-6 oligonucleotides to be screened to be able to find an oligonucleotide that inhibits expression with 66-95% efficiency. Baracchini and/or Bennett also do not provide the identity of the computer program referred to in Taylor. Baracchini and/or Bennett further do not teach or suggest how such results may be attained manually. I could not practice the methods of selecting target regions described in Taylor, even in view of Baracchini and/or Bennett.

13. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge

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that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: Jun 16, 2004 By: Susan Freier

Susan Freier, Ph.D.

Attachments:

Curriculum vitae of Dr. Susan Freier

CURRICULUM VITAE

Susan M. Freier
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ExecutiveDirector AntisenseLead Identification
ISIS Pharmaceuticals
2292 Faraday Avenue
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EDUCATION:
University of California, Berkeley, California
Ph.D in Chemistry, 1976

Carleton College, Northfield, Minnesota
B.A. in Mathematics, summa cum laude, 1972

AWARDS:
Damon Runyon-Walter Winchell Cancer Fund Fellow (1976-1978)
California Regents Fellow (1974-1976)
NSF Graduate Trainee (1972-1973)

OTHER:
Served on Genome Study Section NIH 1997-2002, Chair 2000-2002

EXPERIENCE:

ISIS Pharmaceuticals San Diego 1990-present

Current title: *Executive Director Antisense Lead Identification*

- Determination of microRNA function in mammals. Includes computational identification of miRNAs and miRNA targets. Functional genomics to characterize miRNA biology and identify therapeutic applications of modulation of miRNA activity.
- Use of antisense oligonucleotides for functional genomics of novel targets. Includes: Computational genomics to characterize target RNAs and their variants, rapid throughput screening to identify active antisense oligonucleotides for novel targets, Q-RT-PCR and microarrays for expression analysis.
- Identification and characterization of novel mechanisms for antisense oligonucleotides. Includes computational genomics to identify mRNA variants, alteration of RNA processing, evaluation of siRNA and miRNA mechanisms.
- Biophysical and biochemical evaluation of novel antisense oligonucleotides. Includes: thermodynamics and kinetics of hybridization to oligonucleotide and large structured targets, evaluation of biochemical properties novel

oligonucleotides, characterization of antisense activity in cell assays, protein-oligonucleotide binding.

- Characterization and screening of combinatorial libraries. Includes: theoretical and experimental evaluation of strategies for deconvolution, high throughput screening of combinatorial libraries, bacterial RNA-protein interactions.

Molecular Biosystems Inc.
San Diego
1986-1990

- Development of non-radioactive DNA oligonucleotide probe based tests for detection of infectious and genetic diseases. Experience in: isolation of DNA from clinical samples, probe design, hybridization optimization, assay simplification, process validation. Includes: development of FDA cleared clinical tests for the direct detection of rotavirus or *Campylobacter* in stool, development of colony filter tests for bacterial identification and *in situ* hybridization tests for detection of virus in fixed tissues, cultured cells or patient specimens.

University of Rochester
Rochester NY
1979-1985

- Postdoctoral research with Douglas H. Turner on nucleic acid structure and dynamics. Experience in: chemical and enzymatic synthesis of oligonucleotides (deoxy- and ribo-), hybridization thermodynamics and kinetics, development of a laser temperature jump apparatus, NMR spectroscopy, computer programming and interfacing to laboratory instruments.

Northwestern University
Evanston IL
1976-1979

- Postdoctoral research with Irving M. Klotz and Richard P. Van Duyne on resonance Raman spectroscopy of DNA-mutagen interactions and resonance Raman spectroscopy of hemerythrin. Experience in: protein isolation, laser Raman spectroscopy.

University of California
Berkeley, CA
1972-1976

- Graduate research on the solution conformation of transfer RNA.
  Thesis title: Studies of Nucleic Acid Chemistry:
  Part I. The Solution Structure of Yeast Initiator Transfer RNA Studied by Oligonucleotide Binding
  Part II. A Chemical Model of Mutagenesis
  Experience in: isolation of tRNA, oligoribonucleotide synthesis, oligonucleotide hybridization, NMR spectroscopy.
  Research Advisor: Ignacio Tinoco Jr.

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Susan M. Freier, Matthew Petersheim, David R. Hickey and Douglas H. Turner, "Thermodynamic Studies of RNA Stability", *J. Biomol. Struct. Dyn.* **1**, 1229-1242 (1984).

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Susan M. Freier, Alison Sinclair, Thomas Neilson and Douglas H. Turner, "Improved Free Energies for G:C Base Pairs", *J. Mol. Biol.* **185**, 645-647 (1985).

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Susan M. Freier, Naoki Sugimoto, Alison Sinclair, Dirk Alkema, Thomas Neilson, Ryszard Kierzek, Marvin H. Caruthers and Douglas H. Turner, Stability of XGCGCp, GCGCYp, and XGCGCYp Helices: An Empirical Estimate of the Energetics of Hydrogen Bonds in Nucleic Acids", *Biochemistry* **25**, 3214-3219 (1986).

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Michael C. Griffith, Lisa M. Risen, Michael Greig, Elena A. Lesnik, Kelly Sprankle, Rich Griffey, John S. Kiely and Susan M. Freier, "Mono and Bis PNA's as Triplexing Agents: Binding and Stoichiometry." *J. Amer. Chem Soc.*, **117**, 831-832 (1995).

Lendell L. Cummins, Steven R. Owens, Lixa M. Risen, Danny McGee, Charles Guinosso, Maryanne M. Zounes, Mike Greig, Elena A Lesnik, Susan M. Freier, Henri Sasmor, Richard H. Griffey, P. Dan Cook, "Effects of Uniform 2' Modification on Oligodeoxynucleotide Hybridization and Nuclease Sensitivity", *Nucleic Acids Res.*, **23**, 2019-2024 (1995).

Timothy A. Vickers, Michael C. Griffith, Kanda Ramasamy, Lisa M. Risen and Susan M. Freier, "Inhibition of NF-κB specific transcriptional activation by PNA strand invasion", *Nucleic Acids Res.*, **23**, 3003-3008 (1995).

Yogesh S. Sanghvi, Laurent Bellon, François Morvan, Tomonori Hoshiko, Eric Swayze, Lendell Cummins, Susan Freier, Nicholas Dean, Brett Monia, Richard H. Griffey and P. Dan Cook, "Synthesis, Biophysical, and Biological Evaluations of Novel Antisense Oligonucleosides Containing Dephosphono-internucelosidic Linkages", *Nucleosides & Nucleotides*, **14**, 1087-1090 (1995).

Elena A. Lesnik and Susan M. Freier, "Relative thermodynamic stability of DNA, RNA and DNA:RNA hybrid duplexes, relationship with base composition and structure", *Biochemistry*, **34**, 10807-10815 (1995).

Jacqueline R. Wyatt, Peter W. Davis and Susam M. Freier, "Kinetics of G-quartet-mediated tetramer formation", *Biochemistry*, 35, 8002-8008 (1996)

Susan M. Freier, Danielle A. M. Konings, Jacqueline R. Wyatt and David J. Ecker, "Mutational SURF: A Strategy for Improving Lead Compounds Identified from Combinatorial Libraries", *Bio-organic and Medicinal Chemistry*, 4, 717-725 (1996).

Danielle A. M. Konings, Jacqueline R. Wyatt, David J. Ecker and Susan M. Freier, "Deconvolution of Combinatorial Libraries for Drug Discovery: Theoretical Comparison of Pooling Strategies", *J. Med. Chem.*, 39, 2710-2719 (1996).

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